Glen Canyon National Recreation Area
Aquatic Resources Management

DNA Test Description

NPS Lake Powell Water Laboratory

This document describes the PCR (Polymerase Chain Reaction) test used in the NPS Lake Powell Water Laboratory for the Quagga mussel DNA findings of 10 Aug and 04 Oct 2012.

The NPS PCR test involves crushing all the planktonic (free-floating) organisms in a sample, and extracting their DNA. After the DNA is extracted, a genetic primer set (Ram et al. 2011 quagga primer set*) is used to make many copies of specific DNA sequences, if they are present. With many copies, we can detect the DNA and know its length. When the length matches mussel DNA, the DNA is sent to Arizona State University, where the actual sequence of DNA nucleotides is read. This sequence is then analyzed by NPS scientists using an on-line DNA library. The sequence is statistically measured for its match to known sequences in the library to confirm if the DNA is from mussels. Details of this process as it was applied to the plankton samples of 10 Aug and 04 Oct follow.

The Lake Powel Water Laboratory is certified through the Utah Department of Health for detection of *E. coli* bacteria for public health beach monitoring. All laboratory procedures, including those in the Molecular Laboratory, are developed to meet the standards set by the National Environmental Laboratory Accreditation Council (NELAC). These standards are adopted by many states as requirements of quality assurance and quality control in certified laboratories. Specific certification for mussel DNA detection is not available from any source; however, demonstrations of capability and proficiency tests are accomplished with partner laboratories when possible. Formal Standard Operating Procedures are developed for all aspects of DNA processing (see the Wahweap Molecular Laboratory Standard Operating Procedure Index at the end of this document).

***Primer details**

Species specificity	Primer name	Primer sequence	Product size
Zebra mussel - *Dreissena polymorpha*	ZQ16S147F	AAGACGAGAAGACCCTATCGAA	236 bp
	Z16S383R	AAACTACTGCGCCAAGGAAG	236 bp
Quagga mussel - *Dreissena bugensis*	QCOI151F	GATAGGTGGATTTGGAAACTGG	417 bp
	QCOI568R	ACGATCAGTTAAGAGCATTGTTAAG	417 bp

Ram, J. L., A. S. Karim, P. Acharya, P. Jagtap, S. Purohit, and D. R. Kashian. 2011. Reproduction and genetic detection of veligers in changing *Dreissena* populations in the Great Lakes. Ecosphere 2(1):art3. doi:10.1890/ES10-00118.1

Details of the PCR

PCR of the samples from 10 Aug and 04 Oct produced DNA that matched the expected length of Quagga mussel DNA from the primer set. The sequence of DNA nucleotides was a strong match for Quagga mussels. This section provides details and results of the test, as well as the results of associated quality controls.

From Laboratory Study Notes:

PCR and Electrophoresis Review: 10AUG12 APM1B and 04OCT12 GCD3B samples were submitted for sequencing to Arizona State University. PCR reactions were #881 and 1021 (the two PCR runs for DNA template 10AUG12 APM1B-F4. PCR reaction #1370 was for DNA template 04OCT12 GCD3B-F1.

All samples had amplicons ~400bp long and were sequenced with the Ram et al. 2011 Quagga primer set.

- Plankton tow 10AUG12 APM1B
  - DNA was extracted; the sample was split into 4 tubes for extraction.
    - There is no evidence of cross-contamination for this plankton tow or any other plankton tows extracted on this day.
      - All other plankton tows extracted on in this batch have been run through PCR 2x; none had any amplification.
      - Two extraction negatives were also made; both have been run through PCR and showed no amplification.
  - PCR's were run.
    - APM1B-F4 amplified in both PCR runs.
    - The other 3 DNA template aliquots from this plankton tow (F1-F3) showed no amplification in either PCR run.
    - There is no evidence of cross-contamination in the PCR runs; no other plankton samples or PCR negatives in these PCR runs showed amplification.
- Plankton tow 04OCT12 GCD3B
  - DNA was extracted. The sample was split into 3 tubes for extraction.
    - There is no evidence of cross-contamination for this plankton tow or any other plankton tows extracted on this day.
      - All other plankton tows extracted in batch have been run through PCR 1x; none had any amplification.
      - Two extraction negatives were made; both have been run through PCR and showed no amplification.
  - A PCR was run.
    - GCD3B-F1 amplified; the other 2 DNA template aliquots from this plankton tow (F2-F3) showed no amplification
    - There is no evidence of cross-contamination in the PCR run; no other plankton samples or PCR negatives showed amplification.

PCR product was purified.

Samples were sent to UAGC sequencing facility (for 10AUG12 APM1B-F4 and 04OCT12 GCD3B-F1).

Low-volume (LVL) sequencing was used both times.

Sequencing results:

10AUG12 APM1B-F4:

The two PCR reactions were sequenced with forward and reverse quagga primers, yielding 4 sequences. All sequence data matched quagga DNA.

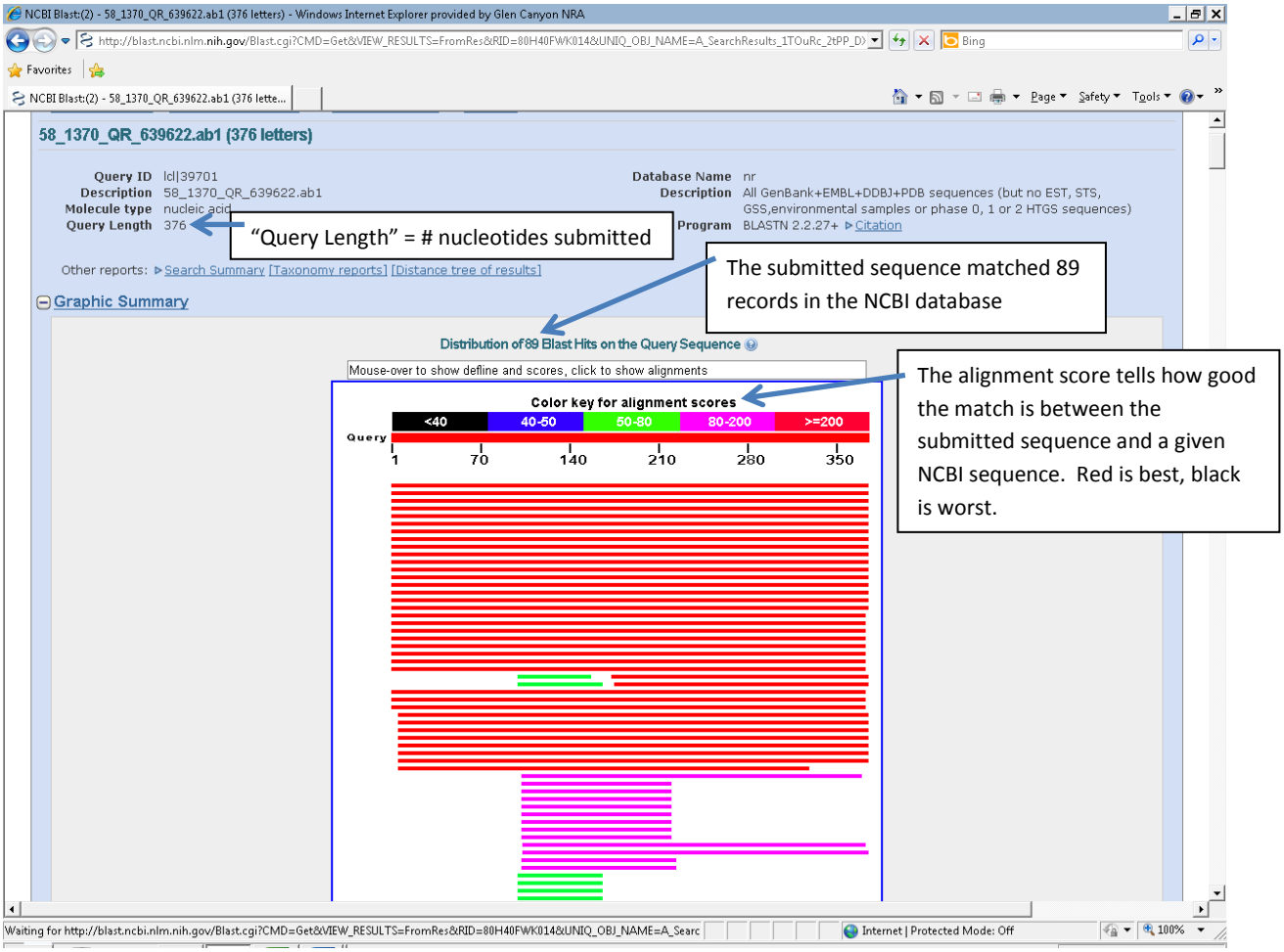
04OCT12 GCD3B-F1:

The one PCR reaction was sequenced with forward and reverse quagga primers, yielding 2 sequences. Both sequences matched quagga DNA.

Interpretation: The 10AUG12 APM1B and 04OCT12 GCD3B plankton tows appear to have collected quagga mussel tissue based on the sequence data. No veligers have been found with microscopy in any APM plankton tows, including the 10AUG12 APM1A replicate tow. One quagga veliger was found with microscopy in the 04OCT12 GCD3A replicate plankton tow.

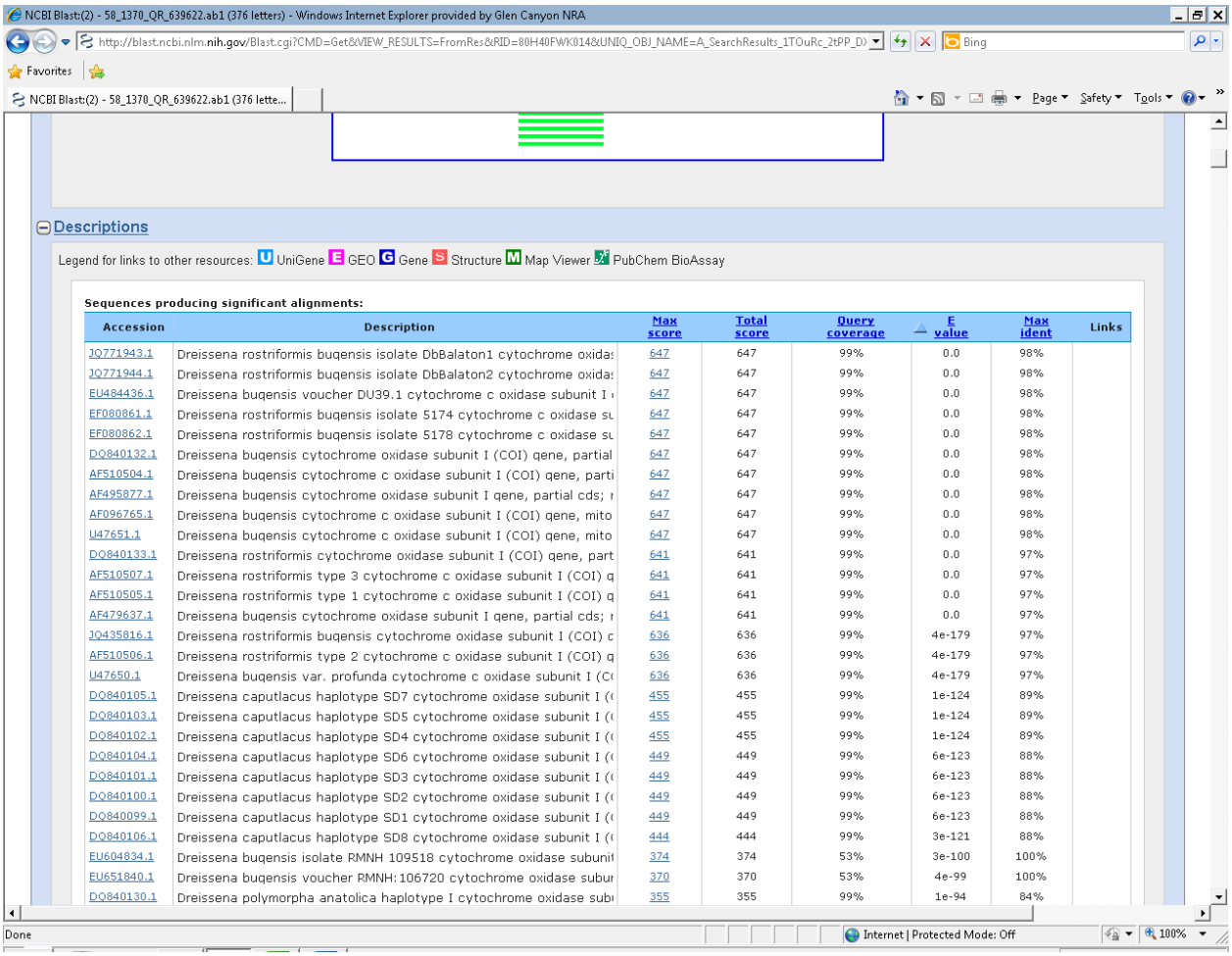
Sequence Match Explanation

Screen captures to explain the analysis of DNA sequence data of October 4 are presented below. 04OCT12 GCD3B-F1 sequence data was input to BLAST (Basic Local Alignment Search Tool - blast.ncbi.nlm.nih.gov/). The screen capture images have been annotated to help explain the results by Sasha Rohde, who oversees the Molecular Laboratory.



The thick red line at the top that has a numbered scale represents the sequence I've submitted – it's called the **Query** by the BLAST program. The thinner lines below represent the 89 NCBI sequences that match my submitted sequence; they're called the **Subjects**.

Some of the NCBI sequences align with all 376 nucleotides I submitted, and so those thin lines extend across the entire length of the Query. Some NCBI sequences only align with part of the 376 nucleotides I submitted, and those thin lines only extend across part of the screen. The best matches are going to be thin red lines that extend across the entire Query.

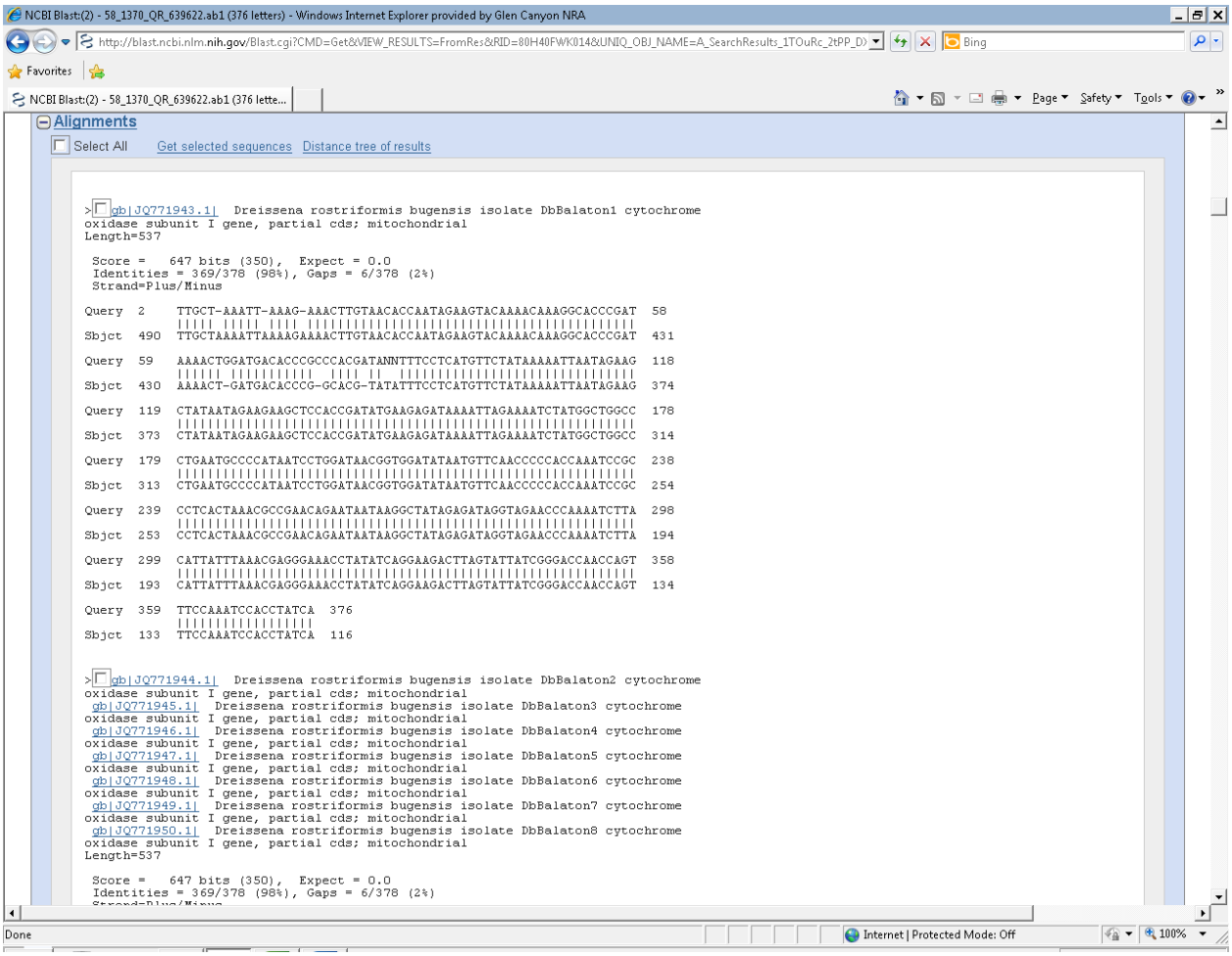


Below the Graphic Summary are the Descriptions of each match; it tells what NCBI sequences matched my submission (the Query) and how good the match was. The best matches are at the top of this section. I record relevant data from this section after each BLAST.

The Accession is a unique identifier NCBI gives to every sequence submitted to their database. The Description describes the NCBI sequence; it includes the species and gene names, if known.

The Query coverage tells how much of the Query aligned with the NCBI sequence. The Max Ident(ity) tells how many of the submitted nucleotides exactly matched the NCBI sequence's nucleotides. The Max Score is calculated based on the number of nucleotides that were identical matches, the number that did not match, and the number of times a gap had to be made in the sequence to maintain the alignment; the Max Score is the alignment score shown in the Graphic Summary. The E value represents the probability of getting a match with *xx* max score in a database the size of the one searched by chance.

A good match will have high Max score, Query coverage, and Max Identity values. It will have a low E value.



Below the Descriptions are the Alignments; they show the nucleotide-by-nucleotide match between the Query (my submitted sequence) and a given Subject (the NCBI sequence).

The Accession number and Description are given at the top. Below that are the alignment score, the E-value ("Expect"), and Identities. Also given are the Gaps and the strand orientation. Gaps tell how many times BLAST had to create a gap in either the Query or Subject's nucleotide sequence to make the alignment; gaps may represent the insertion or deletion of a nucleotide in the Query DNA sequence. A good match will have few gaps. Strand orientation has to do with the double-stranded, complementary (A matches T, C matches G) nature of DNA.

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Standard operating procedures are available. Contact Erin Janicki, Laboratory Director (erin_janicki@nps.gov)